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Tichans.

(54) Low-molecular pectin, and food and drink which contain low-molecular pectin

- (57) A novel pectinase for degradation a pectin or pectic acid is disclosed wherein
 - (1) the novel pectinase is an endopolygalacturonase produced from a genus Saccharomyces,
 - (2) the optimal pH is 4.0,
 - (3) the stable pH range is 4.0 to 8.0,
 - (4) the optimal temperature is 45°C,
 - (5) the enzymatic activity is stable up to 45°C, and
 - (6) the molecular weight is 38,000. A low-molecular pectin having a low viscosity and a high solubility and maintaining the physiological activity as the dietary fiber, and food and drink each of which contains 0.01 to 50 wt% of the low molecular pectin are also disclosed.

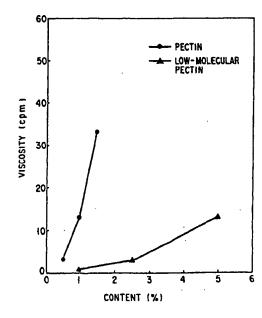


FIG. 5

Description

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The present invention relates to a low-molecular pectin converted from a pectin while the physiological activity of the pectin as a dietary fiber is maintained, and food and drink which contain the low-molecular pectin.

Dietary fibers are defined as hard digestive components in foodstuffs which cannot be digested by human digestive enzymes. The dietary fibers include non-digestive organic materials such as chitin and chitosan in addition to plant cell wall components such as cellulose, lignin, and pectin. In recent years, these dietary fibers are found to have various activities such as a defecation improving effect and an activity of reducing the cholesterol content of blood and to play an important role in preventing diseases of adult people.

Of these dietary fibers, pectic substances such as a pectin and pectic acid have a strong activity as the dietary fibers. Various effects such as a defecation improving effect, an effect of repressing the level of the cholesterol content of blood, an effect of repressing formation of gallstones, and a hypertensive repression effect have been reported. Conventionally, pectic substances have been used as stabilizers in jams, fruit jellies, yoghurt drinks, and lactic acid beverages in food industries. Since the pectic substances have the above effects, they are expected as dietary fibers to be added in food and drink.

A pectic substance is bound with the cellulose in an unripe fruit or plant to be present in the form of a complex called a protopectin. In particular, the protopectin is contained in citrus fruits, apples, and chinese quinces in large amounts. Although this protopectin is insoluble, it is hydrolyzed to produce a soluble pectin or pectic acid when the fruit is ripened.

Of these products, the pectin is a polysaccharide containing galacturonane as a polymer of galacturonic acid as a major component and small amounts of rhamnose, arabinose, xylose, and galactose and having a molecular weigh of 200,000 or more.

The pectin generally has a low solubility and a high viscosity and tends to gel. For this reason, although the pectin has the various effects as described above; only a small amount of pectin is added to food and drink, and it is difficult to add the pectin in food and drink in an amount enough to expect the activity of the dietary fiber.

It is, therefore, the first object of the present invention to provide a low-molecular pectin which has a high solubility and a low viscosity and maintains the physiological activity as the dietary fiber.

It is the second object of the present invention to provide food and drink which contain the low-molecular pectin.

In order to achieve the above objects of the present invention, a pectin is degraded using a pectinase to obtain a low-molecular pectin which has a low viscosity and a high solubility. The present inventors made extensive studies on many pectinase on the basis of the above assumption. As a result, the present inventors found that endopolygalacturonases (EC3. 2. 1. 15) derived from a yeast (i.e., Kluyveromyces fragilis, JTF-1) belonging to the genus Kluyveromyces, a yeast (i.e., Geotrichum candidum, JTF-2):belonging to the genus Geotrichum, a yeast (i.e., Candida Kefyr, JTF-3) belonging to the genus Candida, and a yeast (i.e., Saccharomyces bayanus, JTF-4) belonging to the genus Saccharomyces were suitable as pectinase. In addition, the present inventors also found that even if enzymes obtained from the above yeasts were caused to act up to the degradation limit, the decrease in molecular weight of the pectin by degradation was stopped at the molecular weight of about 20,000; and degradation no longer progressed. The present inventors also found that low-molecular pectins having molecular weights of 20,000 could be obtained by appropriate reaction condition control.

The present inventors have deposited the micro-organisms for producing the pectinase (endopolygalacturonases) used in the present invention designated as JTF-1 (accession number: FERM BP-4056) on October 11, 1991, JTF-2 (accession number: FERM BP-4057) on December 19,01991/JTF-3 (accession number: FERM BP-4058) on March 6, 1992, and JTF-4 (accession number: FERM BP-3916) on July 9, 1992 with the Fermentation Research Institute, Agency of Industrial Science and Technology located at 1-3, Higashi 1-chome, Tukuba-shi, Ibaraki-ken 305, Japan in accordance with the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedures.

The present inventors found for the first time that JTF-4 produced an endopolygalacturonase.

Furthermore, a novel pectinase having the following natures (i) to (vi) is provided:

- (i) The novel pectinase is an endopolygalacturonase which is produced from the genus Saccharomyces and degrades the pectin and the pectic acid.
- (ii) The optimal pH upon reaction at 35°C for 20 minutes is 4.0.
- (iii) The stable pH range upon heating at 35°C for 60 minutes is 4.0 to 8.0.
- (iv) The optimal temperature upon reaction at a pH of 5.0 is 45°C.
- (v) The enzymatic activity upon heating at a pH of 5.0 for 60 minutes is stable up to 45°C.
- (vi) The molecular weight is 38,000.

According to the first aspect of the present invention, the endopolygalacturonases (the endopolygalacturonases produced from the yeasts JTF-1 to JTF-4 will be referred to as JTFP-1, JTFP-2, JTFP-3, and JTFP-4, respectively) pro-

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duced from JTF-1, JTF-2, JTF-3, and JTF-4 are caused to act on pectins to obtain low-molecular pectins. According to the second aspect of the present invention, there are provided food and drink which contain 0.01 to 50 wt% of the low-molecular pectin. State to whether it This invention can be more fully understood from the following detailed description when taken in conjunction with The end of the following and the end of the end of the accompanying drawings, in which: នារស់ព**ា**ស់ដែរតែការស្វាស់មានសម្រាប់ 2 St & 18 THE STATE OF THE PARTY OF THE PARTY OF THE PARTY OF THE Fig. 1 is a diabh showing the relative activity and the pH to determine an optimal pH of an enzyme (JTER-4); Fig. 2 is a graph showing the relative activity and the pH to determine a stable pH range of the enzyme (JTFP-4); Fig. 3 is a graph showing the relative activity and the temperature to determine an optimal temperature of the enzyme (JTFP-4); 5 % % 1.8 5 % box, odo+), box same is an box seminadus oddycin, - this is a same you Fig. 4 is a graph showing the relative activity and the temperature to determine a stable temperature range of the enzyme (JTFP-4); and the water second of the calculation of the calculation for 500 Mill, 21 (d. Rept.) 3 Fig. 5 is a graph showing a viscosity curve of a low-molecular pectin according to the present invention, ลา ธิวกราบบังเติมประธานาธิบาย ชาษ pertic substant as รากา การของค่อสื่อตริเทิกา ลาย อลุโดยมาราบบัง The present invention will be described in detail below. 15 At other water to An endopolygalacturonase (UTFP-4) derived from JTF-4 belonging the genus Saccharomyces according to the present invention will be described below. 305 30 to 10 to 1 JTFP-4 as the enzyme of the present invention acts on a pectin and pectic acid to hydrolyze them. JTFR-4 has the following physicochemical properties: 30 600 auto, aspires and on some according to 944 % L #8661 tufficern is the scheep cobalegions resign to the substitution of the significant (1) Substrate Specificity A 10 000 000 et and tends to get For this reacon, afting the o the permit strategistics by secticiting and arrival JTFP-4 according to the present invention degrades the pectin and pectic acid, but does not degrade soluble starch, dextrin, and xylan, visitable and to visitins and there is no line as a finite of a remaining and the rehas interesting an acceptable present overflor of provide a low-mole, dar pecun which has a high given our this allow viscosity and previous the provided activity as the detary fiber. (2) Optimal pH it is the second cuted of the present intention to produced and drink which contain the low-molecular, reco TTP-4 according to the present invention has an optimal phoneana pH of 4 to expose entremy of reproducto so it is not some and reversing and a recently and a configuration of the same of the property of the configuration of the configura (3) Stable pH Ranger to a contract the sense of the supplier As a resolution of the experience of the tiases (202 filth in a vectoring yeast (i.e. <u>Klusverom ces tragilis</u>, JTP-1, belonging to the group in the En JTFP-4 according to the present invention is stable in the pHigangeof 4 to 8. missings 1 10 (10 per 10 per บลโด กฎที่กุก to the ge <u>in Candida,</u> and alyeast (i.e., <u>Saccharomyces bayanus,</u> JT∺-4) belonging to the <u>genus-Se ชอ</u> ชอ ences were suitable as pedinase. In addition, the present inventors also found that even it end virious distinctions (4) above yeasts well, wilked to act up to the degradation limit, the decrease in motechar weight δ the preci The enzymatic activity of JTFP 4 is 38.9 units/mg protein (number amount of enzyme for producing deumolof a reducing group of the hydrolysate per minute at 35°C in the hydrolysis of pectic acid) be amount of all and all and appropriate black in construction control. (5) Optimal Temperature 1971 1966, and principles of straining operations that between the straining of the is set in the pursuance of the dissignation as JTF if (accussion number) FERIA BEACH (and the pursuance) 40 JTFP-4 according to the présent invention has an optimal temperature near 45°C. The High has reported to the présent invention has an optimal temperature near 45°C. CANC, AFA CONTROL OF BURDER HEBAN EPISME, ON JUN 3, 1934 W. CONTROL OF THE (6) Stable Temperature and a country accounts at any or an attention to be about voolboards and increased for a substitution of the substitution o considerable of this entire to Theaty on the International Necognition of the Cerusary of the Cerusary JTFP-4 according to the present invention is stable until 45°C. 45 구마 (. · · · . . .). Elmana in Norden, el the control of the master of the three periods are a second to the control of (7) Influence of Metal Ion and Inhibitor (4.3) A State of the growth of the notice of the state JTFP-4 according to the present invention is inhibited by 69% with barium chloride, but is not inhibited with magnesium sulfate. TO DESCRIPTION OF THE STATE OF (8) Molecular Weight The molecular weight of JTFP-4 is 38,000. 55 (9) Amino Acid Composition JTFP-4 according to the present invention has a maximum content of glutamine and glutamic acid in a molecule

(130 residues per molecule).

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According to the present invention, the endopolygalacturonase is caused to act on the pectin to obtain a low-molecular pectin.

The endopolygalacturonases generally exist in bacteria, yeasts, fungi, and higher plants. Many steps are required to purify the enzyme from these sources. That is, cells are removed from a culture solution containing microorganisms or the like to obtain a culture supernatant. The culture supernatant is subjected to ammonium sulfate-precipitation to salt out only a protein. The protein is separated based on charges thereof using an ion exchange material. The enzyme is separated by gel filtration in accordance with molecular weights, thus purifying the endopolygalacturonase in accordance with such a general enzyme purification process.

According to the present invention, when a commercially available pectinase is used, purification must be performed to eliminate pectin esterase and hemicellulase from the pectinase.

Since JTFP-1, JTFP-2, JTFP-3, and JTFP-4 produced from JTF-1; JTF-2, JTF-3, and JTF-4, respectively, are extracellular enzymes secreted outside the microorganisms, the culture supernatant can be directly used as a crude enzyme solution. The culture supernatant can be generally obtained such that the yeast is cultured on an agar slant and is then cultured in mass production. The resultant cultured product is centrifuged to eliminate the microorganisms. In this manner, the culture supernatant obtained using the yeast can be directly used in an enzymatic reaction, thereby advantageously simplifying the enzyme purification process.

The culture supernatant is preferably subjected to a simple treatment such as dialysis, ultrafiltration, ion exchange, or gel filtration to eliminate the yeast smell produced in the reaction using this enzyme and to obtain a more transparent solution.

A low-molecular pectin is obtained such that the purified product, culture supernatant, or its treated product of the endopolygalacturonase obtained as described above is reacted with a suspension obtained by suspending a pectin in a buffer solution such as acetic acid.

The type of endopolygalacturonase used in the present invention is not limited to a specific one if it reacts with a pectin to produce a low-molecular pectin having a molecular weight of about 20,000 to 80,000. However, JTFP-1 to JTFP-4 free from laborious operations such as enzymatic purification are preferably used.

As a pectin used in the present invention, any pectin material can be used, and its origin is not limited to a specific one. Therefore, generally known pectins originating from fruits, such as a lemon pectin and an apple pectin can be tised interior. If a to make the origination of the best used in the present of the origination of the pectin can be tised interior.

ो । পাদিa reaction between the pectin and one of चTFP-1 to चTFP-4, a purified product, a culture supernatant (crude i enzyme solution), or its treated product may be used to react with the pectin. ভাষা বিভাগ সমূদ্

The degradation reaction by the enzyme is preferably performed for a reaction time of 12 to 48 hours when the content-of the yeast culture supernatant is 5 to 20 parts by weight with respect to 1 part by weight of the pectin. The preferable reaction temperature and pH are those which allow a sufficient reaction and do not inactivate the endopolygalacturonase, i.e., 30 to 50°C and a pH of 4.0 to 8.0.

According to the present invention, evertife the enzymatic reaction is performed at the degradation limit, the degradation of the pectin is stopped when its molecular weight is about 20,000. Therefore, by controlling the reaction conditions such as the reaction time, a low-molecular pectin having an arbitrary molecular weight falling within the range of about 20,000 to 80,000 can be obtained.

Although the low-molecular pectin according to the present invention can have a molecular weight of about 20,000 to 80,000, the molecular weight preferably falls within the range of about 50,000 to 70,000 in view of retention of the physiological activity as the dietary fiber and ease in addition of the low-molecular pectin in food and drink. A low-molecular pectin most preferably has a molecular weight of about 60,000 and the low-molecular pectin in food and drink.

The degraded product of the pectin may be directly dried and used, or may be further treated.

When a further treatment is to be performed, the degraded product of the pectin is purified by dialysis or ultrafiltration to eliminate galacturonic acid and its oligosaccharide in the degraded product and acetic acid used as the buffer solution in the reaction. The purified degraded product is precipitated using an organic solvent such as ethanol or acetone or dried by freeze drying or spray drying to obtain a powder for later applications.

According to the present invention, there are provided food and drink which contain low-molecular pectins of the present invention.

The low-molecular pectin obtained by the above method according to the present invention has a molecular weight falling within the range from that of a polysaccharide such as pectin or agarose to that of an oligosaccharide such as maltooligosaccharide or fructooligosaccharide. Although the low-molecular pectin has a lower viscosity and a higher solubility than those of the original pectin, it has a defectation improving effect as one of the physiological activities of the dietary fiber.

On the other hand, since the low-molecular pectin according to the present invention has the above properties, it can be contained in an amount which allows to maintain the physiological activity as the dietary fiber, i.e., 0.01 to 50 wt%, and preferably 0.1 to 5 wt%, which cannot be conventionally contained, in a variety of food and drink such as

juices, candies, breads, and jams.

The food and drink which contain low-molecular pectins according to the present invention exhibit improved physical properties and an improved palate at the above contents. These physical properties and palate are different from those obtained into case wherein a conventional pectin is added to food and drink.

As described above, since the enzymes (JTFP-1 to JTFP-4) used in the present invention are extracellular enzymes secreted outside the microorganisms, the culture supernatant can be directly used as a crude enzyme solution and in the enzymatic reaction. Therefore, the enzyme canadvantageously simplify the enzyme purification process and easily degraded the pectin into a low-molecular pectin. When the enzymes used in the present invention are caused to act on pectins up to the degradation limit, the decrease in molecular weight of the pectin upon degradation can be stopped at about 20,000, and further degradation cannot progress, according to the characteristic feature of the enzyme. By controlling the reaction conditions, a low-molecular pectin having a molecular weight falling within the range of about 20,000 to 80,000 can be obtained. All years about Artificial bases of the control of th - 1 5 mm3

Since the resultant low-molecular pectin bas a low viscosity and a high solubility and can maintain the physiological activity (e.g., a defecation improving effect) of the dietary fiber; the low-molecular pectin can be easily added in the food 15 and drink in an amount enough to provide the physiological activity as the dietary fiber.

The present-invention will be described by way of its examples, but is not limited thereto.

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Parts and percentage represent parts by weight and wt% throughout the examples, unless otherwise specified.

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Example 1 resignation of some necessarial and with each reflevour of livery assignment in a some impaising opening sin ther solution such as a mile eco.

Method of Culturing JTE-1 to JTE-4 and Preparation of Crude, Enzyme Solutions: Toler of the envisor of the envisor of the Enzyme Solutions.

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eleable to produce a lighter regular before having a non-cular visigm of about 10,000 to the light reference for (1) Method of Culturing JTEst and Preparation of Crude Enzyme Solution as another to an incident and any agent A 4457.

As a pedan Lear in the present invention, any pedin malified be used, and its profit or not printed to your Kluyveromyces tragilis JTF-1 was cultured on the siant of potato sucrose agar (pH of 5,0) at 27°C for 24 hours. The cultured Kluyveromyces fragilis in one platinum loop was inoculated in 50 ml of a medium (pH of 5.0) containing 5% of 30 glucose; 0.2% of ammonium phosphate, 0,1% of potassium primary phosphate; 0.1% of magnesium sulfate, and 0.4% of a yeast extract and was stationarily cultured at 27%C for 3 days. This outlined product was inoculated in 14 of a medium having the same composition as above the culture medium and was stationarily cultured at 27°C for 3 days. The resultant cultured product was centrifuged at:13,000 rpm/dog:19 minutes to eliminate JTF-1, thereby obtaining a culerable reactor tomperature and pH are those which allow a sufficient reactors and distinstingual and

sudopolygalacts on assile, 30 to 50°C and a pH of 4.0 to 8.0. According to the present invention, excluding September 2 and Preparation of Crude Enzyme Solution, and inventional According to the property of the Preparation of Crude Enzyme Solution, and inventional According to the Preparation of Crude Enzyme Solution, and the Preparation of Crude Enzyme Solution and the Preparation and the Preparatio

cation of the μ in is step and when its molecular weight is about 20 000^{-10} energies by configuration of A culture supernatant was obtained following the same procedures as in (1) except that Geotrichum candidum JTF-2 was used in place of Kluyveromyces fragilis JTF-1. bernatiko ildinasi 01 ili oli 100 ga politika Although the later later cetter accossing to the present divention and area of the countries of the control of

(3) Method of Culturing JTF-3 and Preparation of Crude Enzyme Solution desistant in the control of the control

Candida Kefvr JTF-3 was cultured on the slant of potato sucrose agar (pH of 5.0) at 22°C for 3 days. The cultured Candida Kefyr in one platinum loop was inoculated in 50 ml of a medium (pH of 5.0) containing 5% of glucose, 0.2% of ammonium phosphate, 0.1% of potassium primary phosphate, 0.1% of magnesium sulfate, and 0.4% of a yeast extract and was stationarily cultured at 22°C for 3 days. This cultured product was inoculated in 11 of a medium having the same composition as the above culture medium and was stationarily cultured at 22°C for 4 days. The resultant cultured product was centrifuged at 8,000 rpm for 10 minutes to eliminate JTF-3, thereby obtaining a culture supernatant. Same and the contracting the partition of a syr, we apply a

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(4) Method of Culturing JTF-4 and Preparation of Crude Enzyme Solution

Saccharomyces bayanus JTF-4 was cultured on the slant of potato sucrose agar (pH of 5.0) at 28°C for 3 days. The cultured Saccharomyces bayanus in one platinum loop was inoculated in 50 mℓ of a liquid medium (5% of glucose, 0.2% of ammonium phosphate, 0.1% of potassium primary phosphate, 0.1% of magnesium sulfate, and 0.4% of a yeast extract; pH of 5.0) contained in a 200-ml Erlenmeyer flask and was stationarily cultured at 28°C for 3 days. This cultured product was inoculated in 1 tof a medium having the same composition as the above culture medium and contained in a 3-£ Erlenmeyer flask and was stationarily cultured at 28°C for 3 days. The resultant cultured product was centrifuged at 8,000 rpm for 10 minutes to eliminate JTF-4, thereby obtaining a culture supernatant.

Example 2 1.2 1.4

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Method of Preparing JTFP-4

The culture supernatant obtained in Example 1 was filtered through a millipore filter (pore size: 0.45 µm) to perfectly eliminate JTF-4. The culture filtrate was dialyzed overnight in a 0.02 M acetic acid buffer solution (pH of 5.0) at 5°C. About 600 mℓ of the dialyzed culture supernatant were adsorbed in an ion exchange column (S-Sepharose) and were eluted in accordance with a density gradient method using an aqueous sodium chloride solution. Active fractions were collected, and gel filtration column chromatography (Sephadex G-75) was performed using a 0.02 M acetic acid buffer solution as an eluent. This chromatogram exhibited one highly active peaks. The fractions corresponding to the highly active peak were collected and dialyzed overnight in distilled water at 5°C. The dialyzed product was condensed to 5 mt by gel filtration. About 1 mg of a purified enzyme was obtained as a protein from 600 mt of the culture supernatant.

The enzymatic activity (one unit) was determined by measuring the number of reducing groups in the hydrolysate obtained by the enzymatic reaction in accordance with the Somogyi-Nelson method. That is, one unit is an amount of enzyme for producing it µmol of the reducing groups of the hydrolysate per minute at 35°C (the number of produced reducing groups is figured out as an amount of galacturonic acid). As a result of this measurement, the enzymatic activity according to the present invention was found to be 33.9 units/mg protein.

When SDS polyacrylamide electrophoresis was performed using this sample, the sample was detected as a single. band.

്യെട്ടൂന്നാണ് ആരു പ്രവര്ത്തി ക്രിയിരുന്നത്. അവരു വിവര്ഷ്ട് വര്ഷ്ട് വര്ഷ്ട് വര്യായിരുന്നു വര്യായിരുന്നു. വര്യായ in that in this latter in our demonstrate both which for the control of the control early many evidance of the contract of the con

The following experiment was performed to examine the properties of the enzyme (JTFR-4) of the present invenorganismacque, el per escrete, la como ha discolo de abuelle qui se aen o doto que la trota que la besta de la Direction and the contract and a series of the contract matter and the contract and the con

(1) Substrate Specificity

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In order to examine the substrate specificity of the enzyme, reactivity between the enzyme and substrates shown Rinffable It was examined JOAT GLE Dia to Irm St. O.D. herbs elementation in those woods in across to entitle ### Each substrate was added so that the final concentration of a 0.2 M acetic acid buffer solution (pH of 5.0) was set to be 0.2%, 0.1 ml of an enzyme solution was added to 0.15 ml of each resultant solution and was reacted therewith at 35°O for 20 minutes. The presence/absence of the substrate degradation activity was detected by measuring the number of reducing terminals for each substrate. The degradation activity for each substrate is shown in Table 1. A mark o in Table 1 represents a substrate degraded by the enzyme, and a mark x represents a substrate not degraded by the as as a society of the relative activity of the enzyme of the presenting through the was . Smyzneto as

ுக்குக்கார் மால சமைச்சி to about 20% at 85°C. The stable temperature range of this enzy news. "military to விர் Table 1

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our sees there industries the	Substrate Specifity		
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	Starch	х	
	Xylan	x	

As is apparent from Table 1, the enzyme of the present invention can degrade the pectin and pectic acid, but does not degrade a soluble starch, dextrin, and xylan.

(2) Optimal pH

0.02 ml of an enzyme solution were added to 0.23 ml of a McIlvaine buffer solution having a pH of 2 to 7 and containing pectic acid to obtain a final concentration of 0.2% and were reacted therewith at 35°C for 20 minutes. The activity was measured by a Somogyi-Nelson method. Enzymatic activity values were measured as relative activities when the

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maximum activity value was defined as 100%. As shown in Fig. 1, the relative activities obtained by the Somogyi-Nelson method were plotted as a function of the pH to obtain an optimal pH. As is apparent from Fig. 1, the optimal pH of the enzyme of the present invention was near 4.

(3) Stable pH Range

A distance of the second

Buffer solutions were a 0.2 M McIlvaine buffer solution (pH of 3 to 7) and a phosphoric acid buffer solution (pH of 7 to 10). The 4.9 c for a contract of the 4.0 c for a

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→ cen8 startactu3 (f)

0.025 ml of the enzyme of the present invention were added to 0.125 ml of each buffer solution having a pH of 3 to 10 and were treated at 35% for an hour. 0.15 ml of a 0.5 M acetic acid buffer solution (pH of 5.0) were added to the treated solution to adjust the pH to 5.0. Pectic acid/was added to this solution so that the final concentration was adjusted to 0.2%, the resultant solution was reacted at 35°C for 20 minutes, and the activity was measured by the Somogyi-Nelson method. Enzymatic activity values were measured as relative activities when the maximum activity value was defined as 100%. As shown in Fig. 2, the relative activities obtained by the Somogyi-Nelson method were plotted as a function of the pH to obtain a stable pH range. As is apparent from Fig. 2, the enzyme of the present invention was stable within a pH range of 4 to 8 and to time a 100%.

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0.02 m² of the enzyme of the present invention were added to 0.23 m² of a 0.2 M McIlvaine buffer solution (pH of 5.0) containing 0.2% of pectic acid and were reacted therewith at a temperature of 20°C to 80°C for 5 minutes, and the activity was measured by the Somogyi-Nelson method. Enzymatic activity values were measured as relative activities when the maximum activity value was defined as 100%. As shown in Fig. 3, the relative activities obtained by the Somogyi-Nelson method were plotted as a function of the temperature to obtain an optimal temperature. As is apparent from Fig. 3, the optimal temperature of the enzyme of the present invention was about 45°C.

(5) Stable Temperature Range

0.02:ml of the enzyme of the present invention were added to 0.13 ml of a 0.5 M McIlvaine buffer solution (pH of 5.0) and were heat-treated at a temperature of 20°C to 65°C for 60 minutes. After the reaction solution was gooled with ice, 0.1 ml of a 0.5% aqueous pectic acid-solution was added to each treated solution and was reacted the rewith at 35°C for 20 minutes, and the activity was measured by the Somogyi-Nelson method. Enzymatic activity values were activities when the maximum activity value was defined as 100%. As shown in Fig. 4 the relative activities obtained by the Somogyi-Nelson method were plotted as a function of the temperature to obtain a stable temperature range. As is apparent from Fig. 4, the relative activity of the enzyme of the present invention was 73% up to 45°C, but was reduced to about 20% at 65°C. The stable temperature range of this enzyme was limited up to 45°C.

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(6) Influence of Metal Ion and Inhibitor	t an aa ^r
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	proceeds stated 2

Influences of a metal ion and an inhibitor on the enzyme of the present invention were examined.

Each metal ion and the inhibitor in Table 2 were added in $0.15 \, \text{m}\ell$ of a 0.2 M acetic acid buffer solution (pH of 5.0) containing 0.002 m ℓ of the purified enzyme solution to obtain a concentration of 1 mM. Each solution was reacted at 35°C for 5 minutes, and 0.1 m ℓ of a 0.5% aqueous pectic acid-solution was added thereto. The resultant solution was reacted at 35°C for 20 minutes, and an inhibition ratio was calculated using the Somogyi-Nelson method. Results are shown in Table 2. The inhibition ratio is a relative value with reference to a case (0%) in which a metal or inhibitor is not added.

1	1.12	- 1
 Table 2		

Influence of Metal and Inhibitor						
Compound	Concentration (mM)	Inhibition Ratio (%)				
No additive	•	0				
BaCl ₂	1	69				
KC <i>t</i>	1	35				
Pb(CH ₃ COO) ₂	1 1	54				

Table 2 (continued)

Influence of Metal and Inhibitor						
Compound Concentration (mM) Inhibition Ratio (%)						
MgSO ₄	1	0				
FeSO ₄	1	4-12 18 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1				
CaCl ₂	1	i mee ing 31 ja 3 is				

As is apparent from Table 2, this enzyme was most inhibited (74%) by EDTA. The enzyme was inhibited by a barium ion (barium chloride) by 69%. No inhibition was found with a magnesium ion (magnesium sulfate).

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(7) Molecular Weight

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The molecular weight of this enzyme obtained in Example 2 was measured by SDS polyagrylamide electrophoresis to be 38,000.

(8) Amino Acid Composition

as recognitions, in the following of the seading of

The enzyme of the present invention was hydrolyzed with 6M hydrochloric; acid at 105°C for 24 hours. The hydrolyzed was analyzed by an amino acid analyzer (Hitachi, Model 835) to measure the amount of constituent amino acid. The measurement was repeated three times, and a ratio of the amino acid contents was calculated to obtain an amino acid composition. Results are shown in Table 3.

rousu saw file gmaxili io (3) militaryon may an a Table 3

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	lable		
÷	"Amino Asid C	omposition dama has	Make Pergamban bandan Mek
30 shafk bleniform is Micsaze (f) o	Amino Acid	Amino Acid Residue (per molecule)	kperara katili pipilik 17 (4) je boku zirobolik ili ili ili ili
	Asparagine + aspartic acid	15	
	Threonine	กติสูต ^{อน} ารร้น อาศัตร์	independent of Especial Lower
35 നു ഗ്രീ ctrainshupen - ൂന്നക്ക് മ	hSerinecajous enew (s to (A)	ត (្្រែកសង្គេកម៉ែ 43) នាង១៩ឃុំ នេ	1999-07F-4-00 NTGCF-8
	Glutamine + glutamic acid	130	<u>'</u>
	Glycine	37 ആക്രിയിൽ ഒ	Logard के एक का अंधि के प्र
40 Teg. MR 0004 E - 85 f a gersula a Dispensia ara no (più si assoriò	l \ / = i' = = =	17 Josquewserg worthise Paradose	dos miess Habia an in energia
	Methionine	1	\$4. **
	Isoleucine	ruser v. Saak girakersak süs	petit valor i 1954
45	Leucine	. 7	
m, avallam a DHI i in Alla 2000 a Version in Landon (1901 Alla 2000)	Leucine Tyrosine Phenylalanine	lakur skungruskasik erena uemusue ayr garanga 4 austrus	i esperante de la companya de la co La companya de la co
	Lysine	8	
50	Histidine	6	· • • • • • • • • • • • • • • • • • • •
Book Deliver terranski sekolo	Arginine	3	·
	Proline	8	
55	Note) No experiments for detection tophan and cystine.	were performed for tryp-	

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	As is apparent from Table 3, the a acid and is the second largest in serin		o acid residue per molecule	is largest in the glutamine + glutamic	;
5	Example 4				
•	Preparation and Analysis of Lemon Lo	w-Molecular Pe	ectin		
	a) Preparation of Lemon Low-Molecul	ar Pectin		1900 - 19	
10	(1) Preparation of Low-Molecular Pect	in by JTF-1 Cul	Iture Supernatant	leister trussesser	
15	of 4.8), and 1£ of the culture supernat reacted at 40°C for 24 hours. The res dialyzed overnight with respect to 100-freeze-dried to obtain 58/34g of the left (2) Preparation of Low-Molecular Pect	ultant reaction of fold deionized with monitory monitory monitory.	solution was condensed by water of the sample solution.	a rotary evaporator at 60°C and was In addition, the dialyzed product was	;
20 25	culture supernatant prepared in (2) of the cult	Exampled was useen of (cost in by UTF-3 Gul	s usedicuronহ্ণ আন আৰ্থিক ত্ৰিক্তি কিংস্থানিক আন্ত্ৰ ture Supernatant ভিচাল ৮ - এল উন্নয়েই নিজ্	Least was analyzed an and the	
	supernatant prepared in (3) of Example		white.	, as in (1) except that 12 of the contine	٠
* *	(4) Preparation of Low-Molecular Pect		**		
30	70.40g of a low-molecular pectin w supernatant prepared in (4) of Example	e 1 was used.		s as in (1) except that 1ℓ of the culture	i ji
	, b) Analysis of Lemon Low-Molecular F	ectin	paragina + sepamo ao di la aonina	- :	
35~	The lemon low-molecular pectins (4).	obtained in (1)	to (4) of a) were subjected to	toothe following measurements (1) to	3.
	(1) Measurement of Molecular Weight	. :	yane.	re	
40	The main peak of each lemon low filtration column to calculate its molecular sample.	•		analysis using a TSK-G 4000 PW gel 82, Showa Denko) as the standard	
45	(2) Measurement of Ratio of Galacturo	onic Acid to Neu	utral Sugar	•	•
-				** *	

After each lemon low-molecular pectin was perfectly decomposed using Driselase (KYOWA HAKKO), the ratio of galacturonic acid to neutral sugar was measured by HPLC analysis using a Shodex Sugar SH-1821 column (S. Matsuhashi, S. Inoue and C. Hatanaka, Biosci. Biotech. Biochem., 56, p. 1053 (1992)).

(3) Measurement I of Viscosity

A 5% solution of each lemon low-molecular pectin according to the present invention was prepared, and its viscosity was measured using an E type viscometer (Tokyo Keiki, VISCONIC ED Type).

Results in the above measurements (1) to (3) are shown in Table 4.

Table 4

5	- 1 d - 1 d	Low-Molecular Pec- tin by JTF-1 Culture Supernatant	Low-Molecular Pec- tin by JTF-2 Culture Supernatant	Low-Molecular Pectin by JTF-3 Culture Supernatant	Low-Molecular Pectin by JTF-4 Culture Supernatant
	Yield (%)	58.34	_		70.40
	Molecular Weight	6.6 × 10 ⁴ × 1.4%			98 6.6 × 10 ⁴ × 5.
10	Galacturonic Acid : Neutral Sugar	87.7 : 12.4	87.1 : 12.9	86.3 : 13.7	86.5 : 13.5
	Viscosity (cp)	15.97	15.97	15.97	15.97
	Outer Appearance of	yellowish brown	yellowish brown	yellowish brown	yellowish brown
15	5% Aqueous Solu- tion and a contract	r o e 4 viline describe	arī matri benista a	taeq of Jelphow (en	g et a et et et

(4) Measurement II of Viscosity

For straid \$0.0 unum Pagna appara and a training to the first of the properties of Dunumber apparatus from the contract of the The viscosity of the lemon low-molecular pectin obtained in (1) of a) was compared with that of a lemon pectin. The viscosities were measured using an E type viscometer (50 rpm). Results are shown in Fig. 5: The viscosity of the pectin was considerably reduced. Similar results were obtained for other low-molecular pectins of political and of the

er all elit

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(5) Defecation Improving Effect

4-week old SD male rats were fed with a commercial solid feed (oriental yeast solid feed MF) for 4 days and were divided into four groups each consisting of five rats. A feed containing the low-molecular pectin obtained in (1) of a) and components shown in Table 5, and a solid feed were supplied to each group, and the rats were fed for 9 days. The feces of the rats on the ninth day were collected. Results are shown in Table 6. The hardness of the feces by the solid feed was used as a reference. The hard feces are e (negative), and the soft feces are # (positive) # 2.18 (2001) # 2.18 e como enamistreo. As a control, an abject type hard bandy obtained by adding it part of energy by the lated and wins

no grued with the baid candy of by Results are summarged in Table 8 below.

Component	Control Group (g)	Pectin Group (g)	Low-Molecular Pectin Group (g)
Casein	22	22	22
Lard 08	9	9	9
Corn oil	1 ;	1 "(**)	·**(· 1
Mixed Salt	3.5 (theaths	bros se 3.5° estar e	3.5
Mixed vitamin	1	1 "	¹⁴⁺⁷ 1
Choline chloride	0.2	0.2	0.2
Cholesterol	1	, 1	1 1
Bile acid	0.25	0.25	0.25
Pectin ¹)		5	•
Low-molecular pectin ¹)	<u>-</u>	<u>.</u> ·	5
Sucrose	63.3	58.3	58.3

¹⁾ The pectin and the low-molecular pectin were prepared from a lemon p ctin (Wako Junyaku Kogyo).

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- Table 6

		Solid Feed	Control Group	Pectin Group	Low-Molecular Pectin
i i Gu	Softening	0	in the second	+	+

Judging from the above results, the low-molecular pectin prepared using the yeast of the present invention has a feces softening effect and was found to have a defecation improving effect.

Similar results were also obtained for other low-molecular pectins.

Example 5

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Applications of Low-Molecular Pectings (5)

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Applications using the low-molecular pectin obtained in (1) of Example 4 will be described in the following a) to c).

a) 30% Apple Juice

6 parts of 5-time condensed apple juice, 10 parts of granulated sugar, 0.2 parts of DL-malic acid, 0.02 parts of sodium citrate, and 83 parts of distilled water were mixed with dipart of the low-molecular pectin to prepare a 30% apple juice containing 1 wt% of the low-molecular pectins. (inc. 02) retemption acts a containing 1 wt% of the low-molecular pectins.

The juice containing the low-molecular pectin exhibited smooth nector-like physical properties. years bisness as n

b) Hard candy

Accomposition material (Table 3) containing the part of the dow-molecular specting was used, to prepare an apple type hard candy the lates are part of the containing the hard candy the lates are part of the containing the hard candy the lates are part of the containing the hard candy the lates are part of the containing the hard candy the lates are part of the containing the lates are part of the lates are part of

G. Defecation lingroving Errect

Sugar, millet jelly, and water were mixed with each other, and the resultant mixture was heated to 110°C. The lowmolecular pectin dissolved in a small amount of water was added to the above mixture and was boiled down to 147°C.

Citric acid, spices, and a coloring agent were added to and mixed in the boiled down mixture. The resultant mixture was cooled and molded. As a control, an apple type hard candy obtained by adding 1 part of a pectin was prepared and was compared with the hard candy of b). Results are summarized in Table 8 below.

י מגשב:					
	Noledu az Pelini Group (c	Tabl	Scritrol Group	:n:	Compone
	e anne e a la carrica carrica. E pe	Components (p	arts by weight)		i jeti
		Sugar	6	60	5.3.4
i		Millet jelly		40	5.334 (S. 5.5.5)
· ·	;. ;	Apple juice (5-time condens	ed)	2	2 8 ca 24
		Water		17.5	erright worth
		Citric acid	<u>s</u> n	1	produktivnes i di
		Spices		0.1	
	•	Coloring agent	appropriate	amount	,
	•	Low-molecular pectin		1	

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Table 8

Ny sindrona io an A

i		Outer Appearance	Taste	Total Evaluation
<i>j</i> .	1% low-molecular pectin-added candy	properly dispersed	``	very good
	1% pectin added-candy	lump of powder; not properly dis- persed	too sour; strange taste	not satisfactory

When 1 wt% of pectin was added to the candy material, the pectin formed a lump of powder and could not be properly dispersed. However, when the low-molecular pectin was added in the same amount as that of the pectin, the low-molecular pectin could be properly dispersed and facilitated the preparation of candies. In addition, the pectin-added candy was too sour and had a strange taste. However, the low-molecular pectin-added candy tasted good.

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Bread was prepared using a composition material shown in Table 9.

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S-30.5 parts of the low-molecular pectin were dissolved in water in advance, and this aqueous solution was mixed in the material (Table 9) except for a dry yeast. The resultant mixture was charged in a bread case of a Sanyo bread maker (SPM-B1) and the dry yeast was added thereto. The mixture was kneaded and fermented to bake the bread. As a control, bread was baked using the material composition (Table 9) from which the low-molecular pectin was omitted.

്റ് പാര് 6 ൻ മന്യലയ ഒടിയോ നെ പോടി നിന്നു. ratebatom-യന് നകും മല ഒന്ന് പാര്യുന്ന പ്രദ്യാന **Table 9**

	Enzyme Solution Column dTF 6	Components (part by weight)		Comparation of Low-Molecular R
	in Example 4 oxcupilinst a pullure super-	a High protein flour	ariw 250	and aswirds a place in control (1)
30	method of (2) of Exstruie 1 against 800	e Sugaremaido mateme	ua ert 14 17 ml,	កាមស្នាល ស្រស់ អាកាស ស្រុក
	with elst personal it preceives in the method third of 8.8 × 107.	व भूत्री कि काक्ष्य का एक्सर अं	1 (8 A 10 gar) or	A TO JUST Made or and buffer solution
	pht of 6.5 × 107	ie ned a moleculai Wei	วต าธาบดียังกากกร	. if if Eucade 1. The resultantit
		Skim milk	6.8	
	Enzyme Solution Citizined from JTF-4	re Supernatant of Crude	AD SUBJUST	Properties for the second of t
35		•		
	in Example 4 except that a culture suner-			
	method or (4) of Example 1 against 2007	e retant obtained in we	ਪਰ ਭਾਜ਼ੇ 80 ਪ ਾ ਸ	arrend Bujtálego (a seu en en contra
	itione substitution economic the metrological	o arti forecato di haza as	(8.3 to mg inc	au da remagicas a nice Milatori y inci-
	ना वरह है । है	Low-molecular pecting	2.5 _{377 W}	ปฏิทธิเพลด อิทิโวก สู่แกรกรัฐประกับ

The organoleptic test results are shown in Table 10. Bread containing about 0.5 wt% of the low-molecular pectin and bread of the control were almost the same, but the low-molecular pectin-added bread was softer than the control.

(4) List the summeding the selection of the acceptance of the single-congellection of the selection of th

	y	Outer Appearance and Taste	Total Evaluation
	0.5% Low-Molecular Pectin-Added Bread	Slight yeast-like smell; softer than the control; uni- formly baked in brown	good
• •	Control	pleasant smell of bread; uniformly baked in brown	good

The above tests a) to c) were also conducted for the low-molecular pectins obtained in (2) to (4) of Example 4, and similar results were obtained.

Example 6

Preparation of Apple	Low-Molecular Pectin
5.457.6	

Apple low-molecular pectins were prepared from an apple pectin (Wako Junyaku Kogyo) obtained following the same procedures as in (1) of Example 4, using the culture supernatants prepared in (1) and (3) of Example 1. Each of the resultant low-molecular pectins had a molecular weight of 6.6 × 10⁴. with the process of the many first the properties of a pinctic of

Example 7

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Preparation of Low-Molecular Pectin by Culture Supernatant Obtained by Dialysis

when the state of the second control of the second control of the second (1) Preparation of Low-Molecular Pectin Using Culture Supernatant of Grade Enzyme Solution Obtained from JTF-1

in requesione the color of the management and teaching of the management of the management of the color of th A low-molecular pectin was prepared following the same procedures as in Example 4 except that a culture supernatant obtained by dialyzing overnight 1ℓ of the supernatant obtained in the method of (1) of Example 1 against 300ℓ of a 0.025 M acetic acid buffer solution (pH of 4.8) was used in place of the culture supernatant prepared in the method of (1) of Example 1. The resultant low-molecular pectin had a molecular weight of 6.6×10^4 .

Bread was to the lide of the period of their character Table 9

(2) Preparation of Low-Molecular Pectin Using Culture Supernatant of Crude Enzyme Solution Obtained from JTF-2 the material (Table 3) except for eight verse. The issuitant incure was charged in a bread case of a factor or order of

A low-molecular pectin was prepared following the same procedures as in Example 4 except that a culture supernatant obtained by dialyzing overnight 1/ of the supernatant obtained in the method of (2) of Example 1, against 300/ of a 0.025 M acetic acid buffer solution (pH of 4.8) was used in place of the culture supernatant prepared in the method of (2) of Example 1. The resultant low-molecular pectin had a molecular weight of 6.6×10^4 .

(3) Preparation of Low-Molecular Pectin Using Culture Supernation (Crude Enzyme Solution Obtained from JTF-3

A low-molecular pectin was prepared following the same procedures as in Example 4 except that a culture supernatant obtained by dialyzing overnight 1/ of the supernatant obtained in the method of (3) of Example 1 against 300/ of a 0.025 M acetic acid buffer solution (pH of 4.8) was used in place of the culture supernatant prepared in the method of (3) of Example 1. The resultant low-molecular pectin had a molecular weight of 6.6 × 10⁴.

Skim milk (4) Preparation of Low-Molecular Pectin Using Culture Supernatant of Crude Enzyme Solution Obtained from JTF-4

8.6

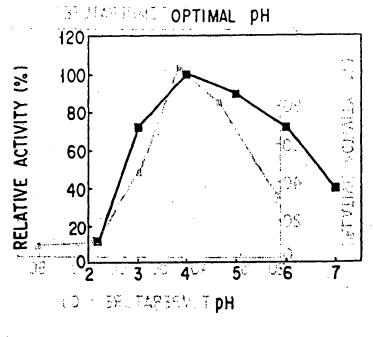
A low-molecular pectin was prepared following the same procedures as in Example 4 except that a culture supernatant obtained by dialyzing overnight 1l of the supernatant obtained in the method of (4) of Example 1 against 300l of a 0.025 M acetic acid buffer solution (pH of 4.8) was used in place of the culture supernatant prepared in the method of (4) of Example 1. The resultant low-molecular pectin had a molecular weight of 6.6 × 10⁴.

Claims

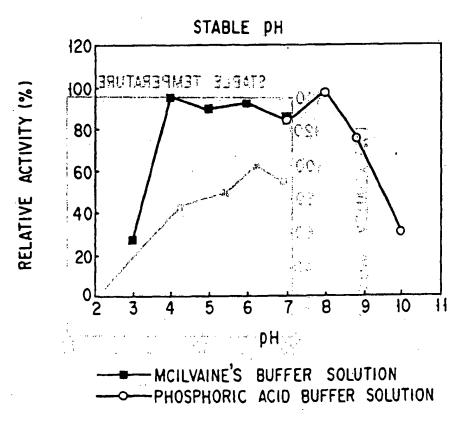
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- the On Kild on cities results are shown in Table 10. Bread course from about 1.5 to 16 or 1. A low-molecular pectin obtained by causing an endopolygalacturonase to act on a pectin are to act on a pectin obtained by causing an endopolygalacturonase to act on a pectin are to act on a pectin obtained by causing an endopolygalacturonase to act on a pectin obtained by causing an endopolygalacturonase to act on a pectin obtained by causing an endopolygalacturonase to act on a pectin obtained by causing an endopolygalacturonase to act on a pectin obtained by causing an endopolygalacturonase to act on a pectin obtained by causing an endopolygalacturonase to act on a pectin obtained by causing an endopolygalacturonase to act on a pectin obtained by causing an endopolygalacturonase to act on a pectin obtained by causing an endopolygalacturonase to act of the act of the
- 2. A low-molecular pectin according to claim 1, characterized in that the endopolygalacturonase is produced from Kluyveromyces, a genus Geotricum, a genus Candida for a genus Saccharomyces. THE MEAN ASSESSMENT OF STREET
 - 3. A low-molecular pectin according to claim 1 or 2, characterized in that the pectin has a molecular weight of 6.6 x 7354 (10g/c) (The Control of the Control of the West Control ind vignici
 - 4. A food or drink characterized by containing the low-molecular pectin according to either one of claims 1 to 3, at 0.01 to 50 weight%. And the second second second

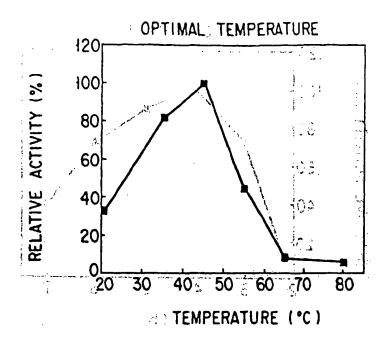
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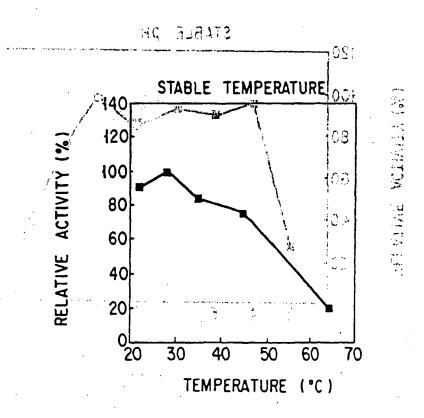
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FIG 5

CONTENT (%)